REVIEW

Myositis autoantibodies: recent perspectives

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Purpose of review
To provide an overview of recent discoveries related to myositis-specific autoantibodies (MSAs) and assays used for their measurement.

Recent findings
New autoantibody specificities have been reported including a MSA directed against eukaryotic initiation factor 3 and a myositis-associated autoantibody directed against heat shock factor 1. The association of anti-TIF1γ with cancer-associated dermatomyositis dependent on age has been confirmed in several large cohorts. Despite MSAs being almost entirely mutually exclusive, several myositis autoantigens are overexpressed in regenerating muscle and do not correlate with the corresponding MSA in any one patient. Further mechanisms may determine the final MSA specificity and are likely to include the need for autoantigen processing and presentation with adaptive T-cell help. The presence of CD4-positive T cells specific for histidyl tRNA synthetase protein in bronchial lavage fluid from antisynthetase patients lends support to this view. Finally, it is widely held that MSA do play an important role in clinical practice among some evidence and concern about commercial assay reliability.

Summary
MSAs continue to provide important tools for clinical diagnosis and management as well as insights into disease mechanisms. Further improvement in the standardization and reliability of routine detection of MSAs is a high priority.

Keywords
autoantibody, autoantigen, diagnosis, myositis

INTRODUCTION

Idiopathic inflammatory myopathies (IIMs) describe a group of disorders characterized by muscle inflammation. IIMs are traditionally described within subcategories: polymyositis, dermatomyositis, and inclusion body myositis. These subtypes categorize IIMs primarily according to the extent of muscle and skin involvement in the disease, but they fail to capture accurately the diversity of clinical symptoms [1]. Autoantibodies are hallmarks of IIMs and are described as either myositis-specific autoantibodies (MSAs) or myositis-associated autoantibodies (MAAs). MAAs can occur in conjunction with another autoantibody and are found in other connective tissue diseases, whereas MSAs are exclusively found in myositis patients and only rarely occur together [2▪]. MSAs are strongly associated with clinical features and can be used to define phenotypic subtypes. The presence of a particular MSA provides important prognostic information with regard to disease development and treatment response. The purpose of this review is to consider recent developments involving MSAs, focusing on the discovery of novel autoantibodies and the development of MSA assays for patient testing.

SUMMARY OF KNOWN MYOSITIS-SPECIFIC AUTOANTIBODIES

Autoantibodies are detected in up to 60% of myositis patients, providing key information relating to associated clinical features and prognosis. MSAs identified to date, and their clinical associations, are summarized in Table 1. Identification of an MSA can increase diagnostic confidence, direct disease management and help avoid unnecessary treatment [2▪,3].

Currently, around 40% of IIM patients will exhibit no detectable autoantibody, providing a

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Curr Opin Rheumatol 2020, 32:000–000
DOI:10.1097/BOR.0000000000000742

KEY POINTS

- Antieukaryotic initiation factor 3 is a novel MSA found in a small number of myositis patients but may be under-recognized in routine screening.
- Although MSA very infrequently coexist in the same patient RNA of several myositis autoantigens are highly expressed in muscle biopsy samples from myositis patients.
- MSA testing is widely used to inform clinical practice despite some concerns about assay performance.

diagnostic challenge that requires alternative tools [2▪]. There is, moreover, limited prognostic information available for this group, potentially complicating disease management and affecting treatment plans. Discovery of novel autoantibodies is therefore crucial to advancing current diagnostic practices and defining new clinical subgroups.

NOVEL AUTOANTIGENS

A novel autoantibody directed against a small protein, eukaryotic initiation factor 3 (eIF3), has been identified in a small number of myositis patients, with no other MSAs present [4▪]. While occurring in only 0.44% of patients, anti-eIF3 appears associated with mild myopathy, absence of cancer or interstitial lung disease and a favourable response to treatment. Notably, anti-eIF3 yields weak or absence of nuclear staining and fine cytoplasmic speckling on indirect immunofluorescence so may be overlooked on routine screening. Therefore, the anti-eIF3-positive patient group may be at high risk of misdiagnosis which would result in under-representation in observational cohorts of myositis patients. Of interest eIF3 plays a key role in skeletal muscle regulation.

Another recent finding has been the finding of autoantibodies directed against heat shock factor 1 (HSF1) by screening a human protein microarray and confirmation using a combination of other assays [5▪]. Anti-HSF1 was present in 11% of IIM patients from a large Chinese cohort and more prevalent in cancer-associated myositis (CAM) (17.2%) compared with non-CAM patients (7.5%). Levels of anti-HSF1 also seemed to mirror disease activity in noncancer patients. Consistent with what has been found with some other myositis autoantigens, expression of HSF1 was increased in regenerating muscle cells of myositis muscle tissue.

Table 1. Currently identified myositis-specific autoantibodies and their clinical associations

Myositis-specific antibody	Antigen target	Frequency in patients	Clinical associations
Anti-ARS (anti-Jo1, anti-PL7, anti-PL12, anti-EJ, anti-HA, anti-OJ, anti-KS, anti-Zo)	tRNA synthetase (histidyl, threonyl, alanyl, glycyl, tyrosyl, isoleucyl, asparagyl, phenylalanyl)	Adult 20–30% Juvenile 2%	Antisynthetase syndrome ILD mechanic's hands Raynaud's phenomenon arthritis
Anti-MDA5	Melanoma differentiation-associated protein 5	Adult 1–30% Juvenile 7%	Rapidly progressing ILD DM typical skin rashes Amyopathic IIM
Anti-HMGCR	3-Hydroxy-3-methylglutaryl CoA reductase	Adult 6% Juvenile 1%	Immune-mediated necrotising myositis Severe muscle disease
Anti-TIF1γ	Transcription intermediary factor 1γ	Adult 7% Juvenile 18–30%	Malignancy in adults DM typical skin rashes Cutaneous photosensitivity 'Red-on-white' lesions
Anti-Mi2	Nucleosome remodelling deacetylase complex	Adult 5–10% Juvenile 4–10%	'Classic' DM with typical skin rashes
Anti-SAE	Small ubiquitin-like modifier activating enzyme	Adult 3% Juvenile 1%	DM typical skin rashes Later muscle involvement
Anti-NXP2	Nuclear matrix protein 2	Adult 2–17% Juvenile 15–20%	Severe onset muscle disease DM typical skin rashes Malignancy
Anti-SRP	Signal recognition particle	Adult 2–6% Juvenile 2%	Immune-mediated necrotising myositis Severe muscle disease Cardiac involvement

ARS, aminoacyl-tRNA synthetase; DM, dermatomyositis; EJ, glycyl-tRNA synthetase; HA, tyrosyl-tRNA synthetase; HMGCR, 3-hydroxy-3-methylglutaryl CoA reductase; IIM, idiopathic inflammatory myopathy; ILD, interstitial lung disease; Jo1, histidyl-tRNA synthetase; KS, asparagyl-tRNA synthetase; MDA5, melanoma differentiation associated protein 5; NXP2, nuclear matrix protein 2; OJ, isoleucyl-tRNA synthetase; PL12, alanyl-tRNA synthetase; PL7, threonyl-tRNA synthetase; SAE, small ubiquitin-like modifier activating enzyme; SRP, signal recognition particle; TIF1γ, transcription intermediary factor 1γ; Zo, phenylalanyl-tRNA synthetase.

However, anti-HSF1 was also found in similar frequency in other autoimmune rheumatic disease controls so does not appear to an MSA.

The same group have reported autoantibodies to poly-U-binding factor 60-kDa protein (PUF60) measured by ELISA in 10.6% (41/388) of Chinese patients with IIM [6], but again not myositis specific as anti-PUF60 was present in a similar frequency in disease controls. PUF60 was initially reported as a novel autoantigen by Fiorentino *et al.* in 2015 [7] with anti-PUF60 present in a range of rheumatic diseases and most commonly found in systemic sclerosis (30%) and dermatomyositis (18%). In the latter study in dermatomyositis anti-PUF60 was associated with the presence of anti-TIF1γ, whereas in the Chinese population anti-PUF60 was detected in clinically amyopathic dermatomyositis (CADM) patients and dermatomyositis patients without currently known myositis autoantibodies. Anti-PUF60 was associated with skin ulcerations and in eight patients followed longitudinally antibody levels declined with disease remission. Several previous studies have suggested a correlation between MSA titre and disease activity especially in relation to anti-MDA5 [8–10] although it is still unclear if antibody titres can provide reliable measurements to assess treatment response.

ANTI-TIF1 SPECIFICITY AND CANCER

The association of anti-TIF1γ and cancer in patients with dermatomyositis is now well established with up to 50% of anti-TIF1γ-positive patients developing an associated cancer within 3 years of myositis onset [11,12]. In a cohort of 263 dermatomyositis cases from the United Kingdom all detected anti-TIF1γ malignancy cases occurred between 3 years prior to and 2.5 years after dermatomyositis onset [13]. Ovarian cancer was particularly common suggesting the need for specific screening strategies. Of interest no anti-TIF1γ-positive case less than 39 years of age developed cancer. The association of anti-TIF1γ and cancer dependent on age was further shown in a large EuroMyositis registry of 1637 patients where the significant association between anti-TIF1γ and CAM only existed for patients more than 58 years of age [2▪]. The lack of an association between anti-TIF1γ and cancer in younger patients with dermatomyositis and indeed in juvenile dermatomyositis suggests that the generation of autoantibodies in this context is not tumour driven and reflects an intrinsically different molecular mechanism, or else that younger patients are unwitting survivors of a tumorigenic event. In a combined Swedish and Spanish cohort levels of anti-TIF1γ declined with successful treatment of cancer [14], indeed suggesting here the cancer itself is driving the autoimmune response.

The IgG subclass of anti-TIF1γ may provide additional prognostic information. In a European cohort, 90% of dermatomyositis patients positive for anti-TIF1γ IgG2 subclass had a positive cancer diagnosis, rising to a 100% predictive value for cancer using a higher cut-off level [15▪]. All cases occurred within 2 years of follow-up.

Antibodies to TIF1γ may also target other members of the TIF1 family of proteins [16]. A small number of patients may target TIF1β alone. Seven such patients (2.4%) were identified from 292 patients positive for at least one anti-TIF1 antibody [17]. Six of the seven had dermatomyositis, two of whom had CADM, one had cancer and none of seven had interstitial lung disease. Although the numbers were small the authors suggested anti-TIF1β may be associated with a milder form of dermatomyositis. Given that all the TIF1 family of proteins have roles in tumorigenesis, future investigation of the expression of TIF proteins in various tissues and environments, and the effects of post-translational modification of TIF1 proteins, could provide key insights [18].

MYOSITIS-SPECIFIC AUTOANTIBODY GENERATION

Recent studies are of interest in providing possible insights into how MSAs are generated. First, MSAs are almost always mutually exclusive. In the large EuroMyositis registry only three cases (0.2%) had more than one MSA [2▪]. Therefore, one may expect that the presence of a MSA in any individual patient may correspond to the corresponding autoantigen being overexpressed in the diseased tissue. However, in a RNA sequencing study of 106 muscle biopsies of IIM patients the increased expression of a given autoantigen in myositis muscle was not associated with autoantibodies recognizing that autoantigen [19▪▪]. Instead most myositis autoantigens were highly expressed and correlated with marker of muscle regeneration. Consequently, there is likely to be other prerequisites in addition to the actual myositis autoantigen expression that determines the MSA specificity.

One important mechanism that may influence the generation of a MSA response and overcome self-tolerance is the presence of T-cell help. In this regard the finding of CD4+ cells in bronchial lavage fluid from three of four antisynthetase patients fluid responsive to histidyl-tRNA synthetase (HisRS) protein (HisRS) is notable [20▪]. Peripheral blood monocytes also responded to HisRS protein in the majority (10/14) of antisynthetase patients, although such a response was also seen in a number of controls.

Together with identifying the presence of anti-Jo-1 antibodies in bronchial lavage fluid, the authors surmised that immune reaction to HisRS protein may occur in the lungs of patients with antisynthetase syndrome.

Finally, in an epitope mapping study utilizing the microbial and autoantigen repertoire of 20 dermatomyositis patients positive for anti-TIF1γ versus 20 controls [21]. Three linear epitopes of six amino acid length were highly specific for SARS-CoV-2. One of the epitopes 'DDAVVC' in the RNA-dependent RNA polymerase protein is a highly ranked CD8 T-cell predicted epitope. The authors speculate that latent exposure to the *Coronaviridae* family may possibly be an important trigger for the development of myositis.

RECENT ADVANCES IN MYOSITIS-SPECIFIC AUTOANTIBODY ASSAYS

Developing a reliable MSA detection assay is challenging. Immunoprecipitation is the reference standard test for detection and is often used in the discovery of novel autoantibodies, with confirmation through techniques such as immunoblotting and mass spectrometry. However, immunoprecipitation requires specialist equipment in a professional laboratory, and results can take weeks to be obtained. This limits the utility of immunoprecipitation for routine testing of patient samples, so the development of commercial MSA assays is required. Currently available MSA assays may provide a quick and less expensive option for MSA testing, although the reliability of commercial assays is a concern and MSA assay validation is difficult. A recent survey of members of the International Myositis Assessment and Clinical Studies Group showed that despite the majority of responders having concerns over assay reliability, there was widespread use of assay results informing diagnosis and clinical decision-making [22]. The standardization and validation of commercial MSA assays is a high priority going forward.

Evaluations and comparisons of currently available MSA immunoassays report conflicting results [23▪,24–27]. The inability to detect rare MSAs has raised the issue of whether certain MSAs are underreported. For example, eight anti-tRNA synthetase antibodies (anti-ARSs) have been identified to date as MSAs. Anti-OJ is an anti-ARS antibody which is thought to be present in fewer than 1% of myositis patients [28]. In multiple studies, line and dot blot immunoassays failed to detect anti-OJ despite positive confirmation by immunoprecipitation [29,30]. Similarly, an ELISA system designed to detect anti-ARS antibodies failed to detect anti-OJ [31]. Novel immunoassays which account for the complexity of antigen–antibody interactions, for example, conformational epitopes, may be required to detect rarer, probably under-reported antibodies [32].

The rate of false negatives in the detection of anti-TIF1γ has been reported to be as high as 76% [23▪]. This is consistent across studies evaluating assay performance [24,27]. Anti-TIF1γ is highly associated with malignancy in adult patients, and as such is a key biomarker for cancer diagnosis [2▪,33]. Anti-TIF1γ is reported in 20–30% of juvenile patients, and a false negative result would result in misdiagnosis [3]. The low sensitivity of anti-TIF1γ detection in commercial line blot and dot blot immunoassays indicates that anti-TIF1γ is unable to interact with the denatured antigen, indicating a conformational epitope [23▪,33]. Conversely, recent investigations indicate that cross-reactivity with anti-Mi2 will result in an increased rate of anti-TIF1γ false positive results [34].

A high rate of false positives in MSA testing is a concern as it could mislead patient diagnosis and treatment. Commercial immunoassays have been reported to produce false positive rates as high as 17% [23▪,24–27]. Line blot immunoassays allow for simultaneous testing for multiple MSAs, which increases the efficiency of testing but additionally increases the rate of false positives. As MSAs are mutually exclusive, a positive result for two MSAs may well indicate a false positive result. It has been suggested that false positive rates can be decreased by defining clear, antibody-specific thresholds for a positive result [26,27]. Using more stringent cut-offs in one study, the rate of positive results for anti-ARS antibodies was decreased by almost 30%, with no patient below the cut-off having antisynthetase syndrome at the time of testing [35].

The recent emergence of a novel particle-based multianalyte assay (PMAT) presents an alternative to commercial line and dot blot assays. A number of studies have investigated the reliability of this novel technology [27,36,37]. Comparison of immune line blot assays (ILA), immunoprecipitation and PMAT shows that PMAT results are more closely correlated to immunoprecipitation results than ILA results [27], but variable results are reported between specific antibodies [36]. The use of PMAT as an alternative MSA assay requires further evaluation larger cohorts with an increased number of controls.

CONCLUSION

The detection of an MSA can provide a clear diagnosis and critical prognostic information. Currently, 40% of myositis patients do not have a detectable MSA; the discovery of novel autoantibodies may define new clinical IIM subgroups and allow earlier diagnosis. Study of novel autoantibodies may also shed light on

the pathogenic mechanisms of IIM, facilitating a broader and deeper understanding of the disease. Affordable and reliable commercial testing of MSAs is a high priority; current assays require standardization and greater validation in studies with larger cohorts and more controls. Novel MSA assays to detect MSAs with more complex antigen interactions are also worthy of investigation.

Acknowledgements

None.

Financial support and sponsorship

Funding to support V.R. was provided by the Bath Institute for Rheumatic Disease.

Conflicts of interest

There are no conflicts of interest.

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